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INDUCTION OF THE ACTION POTENTIAL MECHANISM IN SLOW MUSCLE FIBRES OF THE FROG

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SUMMARY

1. The electrical and structural characteristics of 'slow' muscle fibres of the frog were studied in normal and denervated muscles, and in muscles undergoing re-innervation by a mixed nerve containing large and small motor axons.

2. In agreement with previous studies, slow fibres in normally innervated muscles were incapable of producing action potentials.

3. Approximately 2 weeks after the sciatic nerve was transected or crushed, slow muscle fibres acquired the ability to generate action potentials. These fibres were positively identified as belonging to the slow type, because their passive-electrical and ultrastructural characteristics remained essentially unchanged after the operations.

4. The action potential mechanism induced in slow fibres is sodium-dependent, and is blocked by tetrodotoxin.

5. After long-term re-innervation by a mixed nerve, slow fibres lose their acquired ability to generate action potentials, presumably because small motor axons re-establish connexion with the fibres.

6. It is concluded that the action potential mechanism of slow muscle fibres is under neural control, and is normally suppressed by small motor axons.

INTRODUCTION

In the frog there are two distinct types of extrafusal muscle fibres, generally known as 'fast' and 'slow'. One of the main differences between them is that slow muscle fibres are incapable of generating action potentials

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(Kuffler & Vaughan Williams, 1953*a*; Burke & Ginsborg, 1956; Orkand, 1963; Stefani & Steinbach, 1969). A further difference is that fast fibres give only a transient contracture in response to prolonged depolarization, while the slow fibres respond with a sustained contracture (Kuffler & Vaughan Williams, 1953*b*).

The ability of slow fibres to develop maintained contractures is neurally determined, since Miledi & Orkand (1966) have shown that this property is lost when the iliofibularis muscle, containing both fast and slow fibres, is experimentally innervated by the sartorius nerve which normally supplies only fast muscle fibres. A similar loss of contracture is observed when slow fibres are re-innervated after the sciatic nerve has been simply sectioned or crushed. In this case the maintained contracture response is lost only temporarily, for a time which is presumed to correspond to a period during which slow muscle fibres are re-innervated by fast nerve fibres alone (Elul, Miledi & Stefani, 1970).

Since nerve fibres determine the contracture characteristics of slow muscle fibres, it was of interest to see whether the lack of action potentials in slow fibres is also dependent on a neural influence. Even 10 years ago there was a strong indication that this is so, because it was found (R. Miledi, unpublished observations) that all of the many fibres which were examined within the tonus bundle of iliofibularis muscles innervated by the sartorius nerve were capable of generating action potentials. In contrast, fibres lacking the action potential mechanism were commonly found in the normally innervated iliofibularis muscles from the same frogs. It was, however, still possible that 'inexcitable' slow muscle fibres might have escaped detection in those experiments, and it was therefore deemed desirable to return to the problem and aim at a more positive identification of slow fibres. The original result has now been confirmed; moreover, in the course of this work it was found that removal of the motor nerve supply in itself induces in slow muscle fibres the ability to generate action potentials (for a preliminary communication see Stefani & Steinbach, 1968).

METHODS

The experiments were usually made on the iliofibularis and pyriformis muscles of English frogs (*R. temporaria*) of both sexes. After the operations the frogs were kept in tanks with running water at room temperature and fed with a mixture of homogenized liver and cod liver oil once or twice fortnightly (Harris, 1968).

Operations

All surgical procedures were carried out on the right side of the animals under ether anaesthesia. The muscles of the unoperated side served as controls. In some frogs the sciatic nerve was cut in the pelvis (cf. Miledi, 1960). In other animals the

sciatic was firmly crushed, also in the pelvis, with a forceps whose tip was about 1 mm wide. When long periods of denervation were required, the 7th, 8th and 9th spinal nerves were sectioned where they join to form the sciatic, and their proximal ends tied into the musculature of the back, as far as possible from the leg. In addition, the sciatic itself, including most of the iliofibularis nerve, was pulled out (for further details cf. Elul *et al.* 1970).

Intracellular recording

The electrophysiological results reported here were obtained from studies of the iliofibularis and pyriformis muscles. The latter muscle was very useful because slow fibres were easily accessible on its cutaneous surface (Stefani & Steinbach, 1969; Miledi & Stefani, 1970), but similar results were also obtained in slow muscle fibres of the iliofibularis muscle. The pyriformis muscle was also used for some of the morphological studies, since the electron-microscopic structure of its fast and slow muscle fibres is similar to the corresponding type of fibre in the iliofibularis muscle (R. Miledi & E. Stefani, unpublished). Conventional intracellular techniques for recording and stimulating were used. Recording micropipettes were filled with 3 mM-KCl and current-passing micropipettes with 2 M potassium acetate. Current-passing micropipettes were connected via a chlorided silver wire and a 500–1000 MΩ series resistance to a square-pulse generator in parallel with a DC voltage source. The current path was completed by an agar-Ringer/AgCl/Ag half cell in direct contact with the bath, connected through a 500 kΩ monitor resistance to ground.

Single muscle fibres were impaled under visual control through a compound microscope (usually 60 ×), with the recording and stimulating micro-electrodes separated by about 50–200 μm. Usually two to eight slow muscle fibres were examined in each muscle. In some experiments a second recording electrode was inserted in the same fibre at 0·5–4 mm from the first pair, in order to study propagation of potentials.

During the experiments the temperature of the bath containing the isolated muscle was kept at 4–7° C by a Peltier-effect cooling unit attached to the chamber (Katz & Miledi, 1963). Lowering the temperature greatly helped to maintain the electrodes inside the fibre, presumably because it reduced the local contracture which frequently accompanies impalement of slow muscle fibres. The normal Ringer solution had the following composition in mM/l.: NaCl 115; KCl 2·5; $CaCl_2$ 1·8. Sodium-free solutions were prepared by total replacement of NaCl with 115 mM Tris (hydroxymethyl-aminoethane chloride, Sigma). When isotonic Ca solution was used, NaCl was replaced with 80 mM-$CaCl_2$. All solutions were buffered to pH 7·3 with 2·5 mM Tris buffer.

Electron microscopy

Muscles were fixed in 6 % glutaraldehyde in phosphate buffer for periods ranging from 1 to 43 hr. Selected regions of the muscle were then cut out and post-fixed in 1 % OsO_4 for ½–2 hr. After dehydration with ethanol, some blocks were stained with phosphotungstic acid while others were left for staining in sections after embedding in Araldite (CIBA Ltd.).

In two experiments post-operative electrical properties and ultrastructure were examined on the same identified single muscle fibres. When the electrophysiological experiment was ended, a map was made of the region noting the position of nerve branches, blood vessels, melanophores and other landmarks. In addition some neighbouring muscle fibres were damaged. All these landmarks were clearly recognizable after glutaraldehyde fixation and the studied muscle fibres were then identified, teased out and prepared individually for electron-microscopic study.

RESULTS

Action potentials in slow muscle fibres

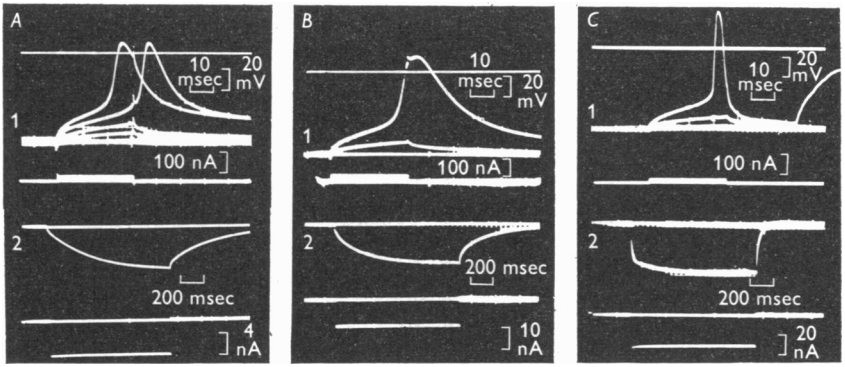
Normally innervated fast and slow muscle fibres can be distinguished because of the different electrical characteristics of their membranes (Burke & Ginsborg, 1956; Adrian & Peachey, 1965; Stefani & Steinbach, 1969). In general, fast fibres have membrane time constants of about 20 msec, while for slow fibres the time constant usually exceeds 200 msec (Stefani & Steinbach, 1969; Miledi & Stefani, 1970). In addition, slow fibres can be identified by raising the external calcium concentration which produces an increase in membrane resistance and time constant that is very much greater than in fast fibres (Stefani & Steinbach, 1969). These differences between fast and slow muscle fibres persist after denervation (Miledi & Stefani, 1970) and may therefore be used to identify them.

The electrical properties of slow muscle fibres were studied in denervated muscles and in muscles undergoing re-innervation after the sciatic nerve had been sectioned or crushed (see Methods). Slow fibres are easily damaged by penetration of the micro-electrodes, resulting in a drop of resting potential of about 10–40 mV from the initially recorded level. Therefore, a steady hyperpolarizing current was passed through the membrane to bring the resting potential (E_{RP}) close to that recorded initially (*ca.* 80 mV); the electrical properties of the slow fibres were generally studied at this membrane potential (E_m).

Approximately 2 weeks after transection or crushing of the sciatic nerve, the slow fibres in the operated side were able to produce regenerative action potentials. Text-fig. 1 illustrates typical responses in two such fibres (*A* and *B*). For comparison, an action potential from a denervated fast fibre is shown in Text-fig. 1*C*. The Figure also illustrates the voltage changes evoked by the square pulses used to identify the fibre types. The difference in time constant of slow (*A*2 and *B*2) and fast fibres (*C*2) is obvious. Another interesting feature, also illustrated in Text-fig. 1, is the slower rate of rise and longer duration of the action potentials in the slow fibres.

In the absence of the steady hyperpolarization, the resting potential of most slow fibres fell to between -40 and -60 mV, and no action potential could be evoked with depolarizing pulses. However, action potentials, though sometimes small in amplitude, could still be generated at the break of a strong hyperpolarizing pulse (see Text-fig. 5*B*1). In normally innervated slow muscle fibres from the iliofibularis (sixty-five fibres examined) and from the pyriformis (fifty-three fibres), action potentials could not be elicited with either depolarizing or hyperpolarizing pulses, not even during a steady conditioning hyperpolarization (cf. also Burke & Ginsborg, 1956).

From these experiments we conclude that after the sciatic nerve is cut or crushed, a population of muscle fibres is found which have passive electrical properties similar to normally innervated slow muscle fibres, but can now produce regenerative action potentials. To characterize the type of fibre concerned more precisely, we turned to electron microscopy.



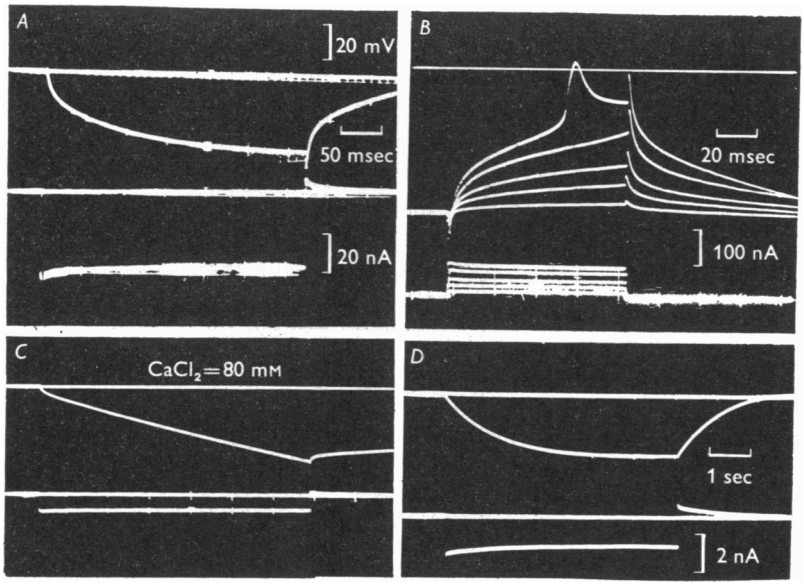
Text-fig. 1. Action potentials in slow fibres from pyriformis muscles. *A* 1: 13 days after crushing the sciatic nerve. *B* 1: 30 days after denervation. *C* 1: action potential in a fast fibre from the same muscle as *B*. *A* 2, *B* 2, and *C* 2: hyperpolarizing potentials used to identify fibre types. A steady hyperpolarizing current was applied to slow fibres in order to bring the membrane potential near to the level recorded immediately after insertion of the electrodes (see p. 740). In these and subsequent figures E_{RP} denotes the resting potential (after inserting all electrodes), and E_m the hyperpolarized membrane potential. E_{RP}: -60 mV in *A*; -65 mV in *B*. E_m: -80 mV in *A*; -77 mV in *B*. Note the slower time course of the action potentials in slow fibres (*A*, *B*).

Ultrastructure of fibres with 'slow' action potentials

Normally innervated fast and slow muscle fibres of the frog differ importantly in electron-microscopic structure (Peachey & Huxley, 1962; Page, 1965; Hess, 1967). For example, slow fibres lack a distinct M-line at the level of the A bands, and have wavy Z-lines with irregular packing of the I filaments. Furthermore, slow fibres lack the regularly arranged transverse tubular system and the characteristic triad arrangement of fast fibres.

When the iliofibularis muscle is re-innervated by the 'fast' nerve fibre of the sartorius nerve, the maintained contracture response is lost, but the characteristic structural differences between fast and slow fibres persist (Miledi & Orkand, 1966). Hence the structural features of the fibres can be used for further characterization of those fibres which, after the operations, show the passive electrical properties of normal slow fibres but are capable of generating action potentials. To this end, the electrical properties and

ultrastructure were examined in identified individual fibres. This was done successfully on two fibres from the iliofibularis muscle: one 32 days after crushing the sciatic, and the other 70 days after transecting it. Both fibres had the 'slow' electrical properties; at the end of the electrophysiological experiment the muscles were fixed, and the fibres were localized, isolated and prepared for electron-microscopic study.



Text-fig. 2. Electrical characteristics and effect of Ca in a slow fibre. Iliofibularis muscle 70 days after transecting the sciatic. *A* and *B*: in normal Ringer. *C* and *D*: in isotonic calcium. E_{RP}: −56 mV in Ringer; −94 mV in isotonic Ca. E_m: −50 mV in *A* and −80 mV in *B*.

The electrical characteristics of one of these fibres is illustrated in Text-fig. 2, obtained while the muscle was in normal Ringer (*A*, *B*) and after soaking the preparation for 10 min in isotonic calcium (*C* and *D*). An action potential is shown in *B* and the characteristic large increase in input resistance and time constant due to the high Ca can be seen from the records in *C* and *D*. Pl. 1 illustrates the structure of the same fibre fixed after treatment with isotonic Ca, which does not affect the basic morphological differences between normal fast and slow fibres. Pls. 2–4 show electron-micrographs from the other isolated fibre which also had 'slow' electrical characteristics and was able to give action potentials. Note, in both fibres, the absence of M-lines, the irregular Z-lines, the wide myofibrils, all characteristics of slow fibres. The tubular system, with narrow longitudinal and transverse tubules, can be seen in Pls. 3 and 4, together

with some transversely arranged fine filaments. It should also be mentioned here that, as in normal slow fibres, triads were seen only occasionally.

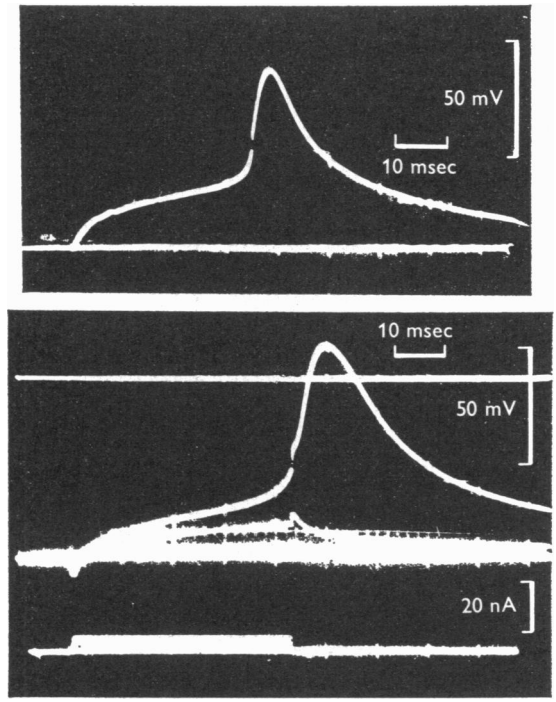
These experiments show conclusively that after the sciatic nerve has been cut or crushed, muscle fibres are found which can generate action potentials and yet possess the ultrastructural and electrical characteristics of normally innervated slow fibres. We are therefore led to conclude that the operative procedures have caused slow fibres to develop the mechanism required for the production of regenerative action potentials.

The development of action potentials in slow fibres

As already mentioned, the two fibres used for the combined electrophysiological and morphological examination were from iliofibularis muscles 32 and 70 days, respectively, after the crushing or cutting of the sciatic. There was no contraction to nerve stimulation in either muscle, but we know from previous work that at these times the maintained contracture response is lost, presumably because the slow fibres have been re-innervated by fast nerve fibres (Elul *et al.* 1970). Thus these results, like the earlier ones on transplanted iliofibularis muscles innervated by the sartorius nerve, suggested that fast axons induce the action potential capability in slow muscle fibres. However, as the experiments proceeded it was found that denervation alone was sufficient to cause this transformation of the muscle fibre membrane.

The evidence for this is that action potentials were observed in denervated slow fibres, from both iliofibularis and pyriformis muscles, after precautions had been taken to prevent re-innervation (Text-fig. 3, and Table 1). Action potentials were also seen after crushing the sciatic, at a time when no regeneration of neuromuscular junctions would be expected (Table 2). A further indication that denervation alone is sufficient to induce the action potential mechanism in slow muscle fibres was provided by preliminary experiments on isolated iliofibularis muscles kept in organ culture conditions (cf. Harris & Miledi, 1966). After 4 weeks in culture, slow muscle fibres were able to generate small action potentials of about 10 mV amplitude.

In denervated preparations, as well as in those where re-innervation was allowed, slow fibres did not require the ability to generate action potentials until about 2 weeks after the operation. The fact that no sign of an action potential was seen in the slow fibres of one muscle examined 11 days after crushing the sciatic, while action potentials were recorded from another muscle, just 2 days later (see Table 2), suggests that the transition of the membrane (from inexcitable to excitable) occurred too rapidly to be detected by our methods of sampling.



Text-fig. 3. Action potentials in denervated slow muscle fibres. *A*: from an iliofibularis muscle 15 days after sectioning and re-routing of spinal nerves plus removal of sciatic (cf. Methods). $E_{RP} = -56$ mV, $E_m = -80$ mV. *B*: from a pyriformis muscle after a similar operation. $E_{RP} = -50$ mV, $E_m = -80$ mV.

Re-innervation of transformed slow muscle fibres

Approximately 4 weeks after the sciatic was crushed, some fast muscle fibres became functionally re-innervated. In the same muscles, slow fibres still gave action potentials in response to direct electrical stimulation; and in one experiment, 34 days after the operation, two slow fibres responded to nerve stimulation with an end-plate potential which could trigger an action potential (Text-fig. 4). This shows that re-innervated slow fibres are capable of full neuromuscular transmission, but unfortunately we are unable to decide whether these slow fibres had become re-innervated by a fast or a slow axon.

It has previously been shown (Elul *et al.* 1970) that, after losing their maintained contracture response, slow muscle fibres re-acquire this property if sufficient time is allowed for regeneration of neuromuscular junctions. It is presumed that during this time slow nerve fibres establish contact with slow muscle fibres and 're-induce' the contracture response.

Similarly, as re-innervation progresses, slow fibres lose their acquired ability to generate action potentials. For example, in one muscle 41 days after the sciatic had been crushed, the two slow fibres examined were incapable of producing full-sized action potentials, although one of them gave a small spike, approximately 8 mV in amplitude, following a strong hyperpolarizing pulse. Again, no sign of an action potential was detected in four slow fibres from a muscle examined 57 days after the sciatic was crushed (cf. Text-fig. 5*A* and Table 2). In contrast, slow fibres denervated

TABLE 1. Development of action potential mechanism in denervated* slow muscle fibres

Days after operation	Muscle	Slow fibres: With action potential	Slow fibres: Without action potential
5·5	Iliofibularis	—	2
15	Iliofibularis	2	2
—	Pyriformis	2	—
16	Iliofibularis	3	—
16	Iliofibularis	1	—
—	Pyriformis	3	4
22	Pyriformis	2	—
30	Pyriformis	4	—
41	Pyriformis	3	—
44	Pyriformis	4	—
47	Iliofibularis	2	—
57	Pyriformis	2	—
49	Iliofibularis	4	—
59	Pyriformis	4	—
178	Iliofibularis	1	—
183	Iliofibularis	3	—

* Spinal nerves cut, sciatic evulsed etc. (cf. Methods).

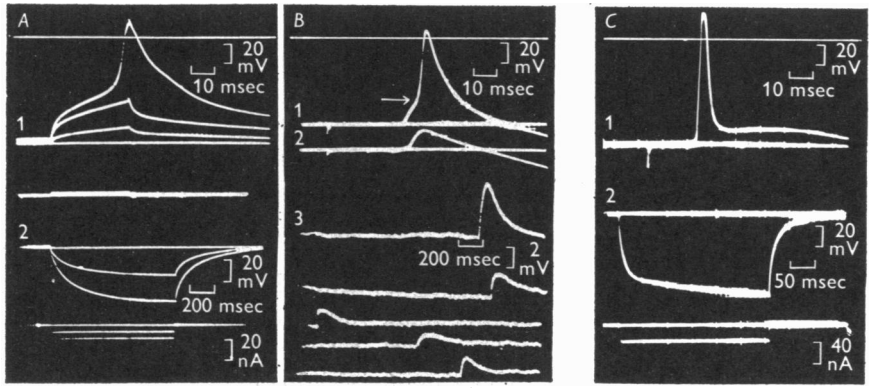
for similar or even longer periods (Table 1) were still capable of generating action potentials (Text-fig. 5*B*). Thus, it appears that the action potential mechanism is lost from slow fibres, some two months after the sciatic has been crushed. It is interesting to note that the maintained contracture response also returns at about this time (cf. Elul *et al.* 1970).

After long-term re-innervation, one year or so subsequent to crushing or cutting the sciatic, most, if not all, of the slow muscle fibres were functionally re-innervated and responded with end-plate potentials to nerve stimulation. These end-plate potentials were composite, indicating that most fibres had received innervation from more than one axon. At this time, slow muscle fibres were incapable of generating action potentials (for further details cf. Text-fig. 9 and p. 220 in Elul *et al.* 1970).

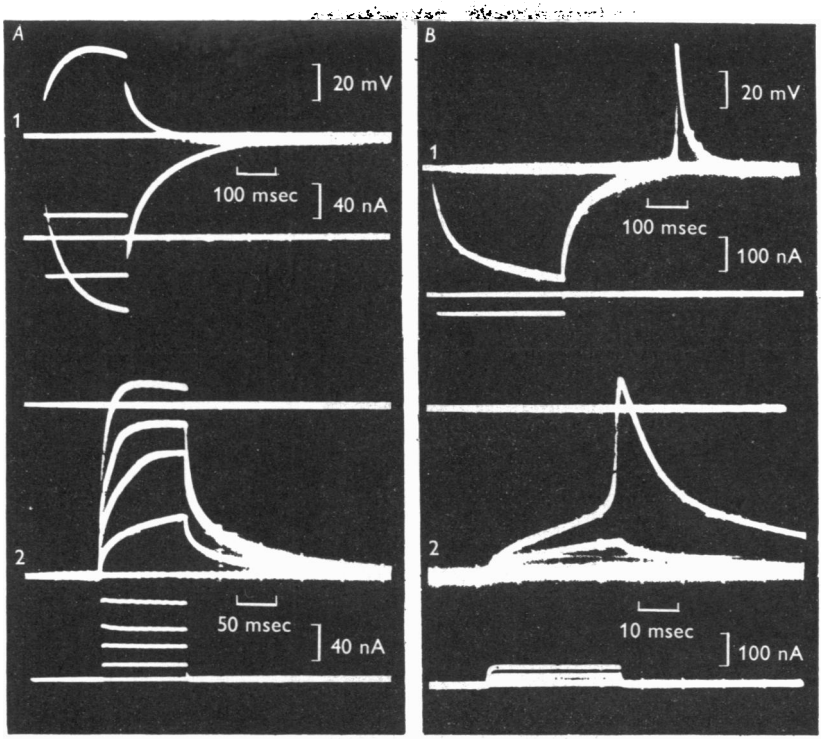
TABLE 2. Development of action potential mechanism in electrically identified slow muscle fibres, after crushing the sciatic

Days after operation	Muscle	Slow muscle fibres: With action potential	Slow muscle fibres: Without action potential	Neuro-muscular transmission potential
2·5	Iliofibularis	—	3	—
6·5	Iliofibularis	—	1	—
8·5	Iliofibularis	—	3	—
11	Iliofibularis	—	3	—
13*	Iliofibularis	3	—	—
—	Pyriformis	6	—	—
14	Iliofibularis	4	—	—
19	Iliofibularis	1	—	+
20	Iliofibularis	5	—	—
21*	Iliofibularis	3	—	—
—	Pyriformis	6	—	—
28*	Iliofibularis	2	—	+
—	Pyriformis	6	—	+
34*	Iliofibularis	1	—	+
—	Pyriformis	4	—	+
37	Pyriformis	5	—	+
41	Pyriformis	1	1	+
57	Pyriformis	—	4	+

* Iliofibularis and pyriformis muscles from same animal.



Text-fig. 4. Action potential in a functionally re-innervated slow fibre of a pyriformis, 34 days after crushing the sciatic. In *A* 1 the action potential was evoked by intracellular stimulation. In B 1 a nerve stimulus elicits an end-plate potential which triggers the action potential (arrow). In *B* 2 the steady hyperpolarization was increased and the end-plate potential became subthreshold. *B* 3: spontaneous miniature end-plate potentials in the same fibre. *C*: action potential, evoked by nerve stimulation, of a fast fibre in the same muscle. E_{RP}: −48 mV. E_m: −102 mV in *A* 1; −78 mV in *A* 2; −84 mV in *B* 1; −104 mV in *B* 2; −78 mV in *B* 3.



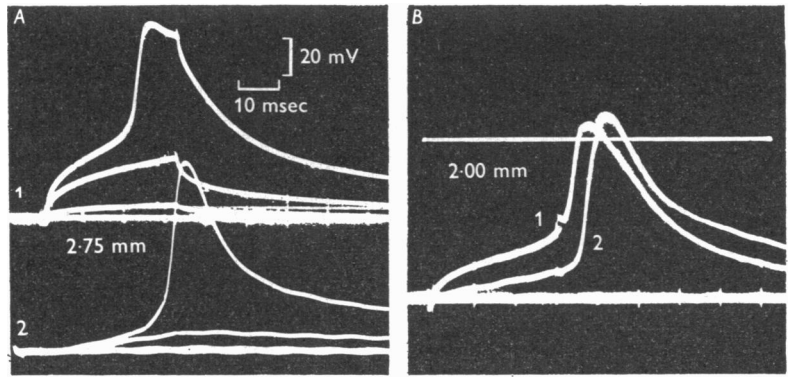
Text-fig. 5. Abolition of the action potential during continued re-innervation. *A*: lack of action potential in a slow fibre from a pyriformis muscle 57 days after crushing the sciatic nerve. *B*: action potentials in a pyriformis muscle from a different frog 57 days after cutting the sciatic. *B* 1: anode-break action potential. *B* 2: action potential evoked by a depolarizing pulse. E_{RP}: −30 mV in *A* and −60 mV in *B*. E_m: −50 mV in *A* 1; −55 mV in *B* 1; −96 mV in *A* 2 and −94 mV in *B* 2.

Some properties of action potentials in slow fibres

Propagation. In six experiments a second recording electrode was inserted into slow muscle fibres and action potentials were recorded simultaneously from the two recording sites. In eight fibres, where a large action potential was seen near the current micro-electrode, an equally large, or even larger action potential was recorded, after a delay, from the more distant electrode. This is illustrated in Text-fig. 6. These experiments show in a qualitative way that the acquired action potentials propagate along slow muscle fibres. Conduction velocities were not measured accurately because the site of origin of the action potential was not always well defined. However, in six fibres, with recording electrode separations of 1·5–5 mm, we estimated a conduction velocity of 0·2–0·6 m/sec at 6° C, which may be

somewhat slower than the conduction velocity of action potentials in fast fibres at a similar temperature.

Amplitude and rate of rise. Action potentials in different slow fibres varied in amplitude and rate of rise. One reason for this may be that slow fibres, due to their high input resistance, are very susceptible to damage caused by insertion of the micro-electrodes. Moreover, contractions are often associated with the action potentials and lead to further damage. Additional complications are introduced by the need to apply a steady hyperpolarizing current in order to maintain the membrane potential near -80 mV. Therefore, in order to measure the amplitude and rate of rise of action potentials,



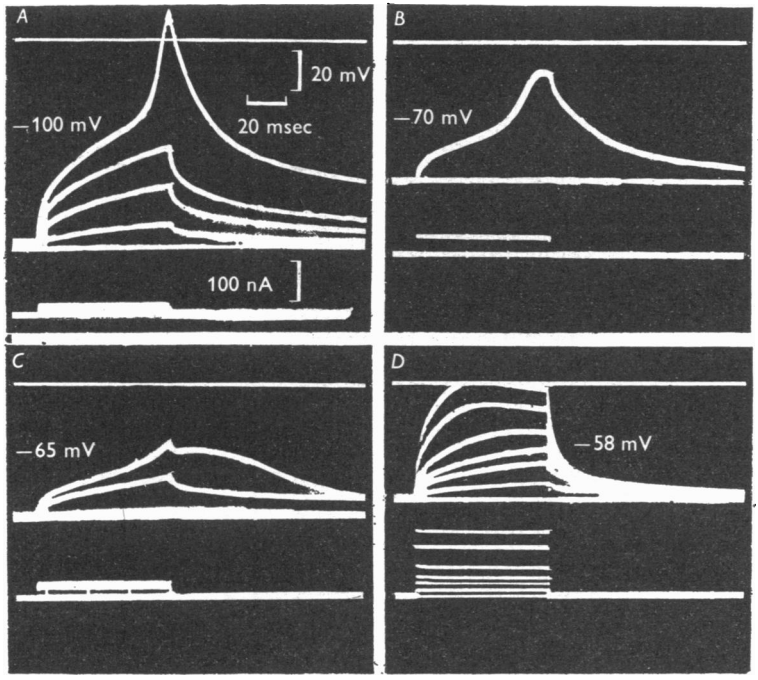
Text-fig. 6. Propagation of action potentials in slow fibres. *A*: from a pyriformis 21 days after crushing the sciatic. *B*: from a pyriformis 30 days after sectioning the sciatic. Simultaneous recording of the action potentials from a point about 50 μm from the stimulating electrode (*A* 1 and *B* 1), and from a more distant point (2·75 mm in *A* 2 and 2 mm in *B* 2). E_{RP}: -65 mV in *A* and *B*. E_m: -100 mV in *A* and -86 mV in *B*.

only propagated action potentials were used because they were recorded away from the stimulating micro-electrodes, thus reducing some of the complications introduced by the depolarizing pulses. Such action potentials in eight slow fibres had amplitudes of 67–94 mV and a maximum rate of rise of about 25 V/sec (see Text-fig. 6). Thus, it seems that the amplitude of action potentials in fast fibres is somewhat larger, and their maximum rate of rise about fourfold faster than in slow fibres (at 6° C). But further experiments are required to determine how far the complications mentioned above distort the measurements, and to explore the possibility that a more complete transformation is effected by prolonged re-innervation with fast axons.

Action potential inactivation at low membrane potentials. As in fast muscle fibres, the amplitude of the action potentials of slow fibres depended on the membrane potential levels at which they were evoked. This can be

seen in Text-fig. 7, illustrating action potentials elicited at different levels. There was a marked reduction in amplitude on changing the membrane potential from -100 to -70 mV, and the regenerative response was reversibly abolished at -58 mV.

Ionic dependence of slow fibre action potentials. It is well known that the action potential of fast muscle fibres in the frog results from an increase of membrane permeability towards Na ions (Nastuk & Hodgkin, 1950),

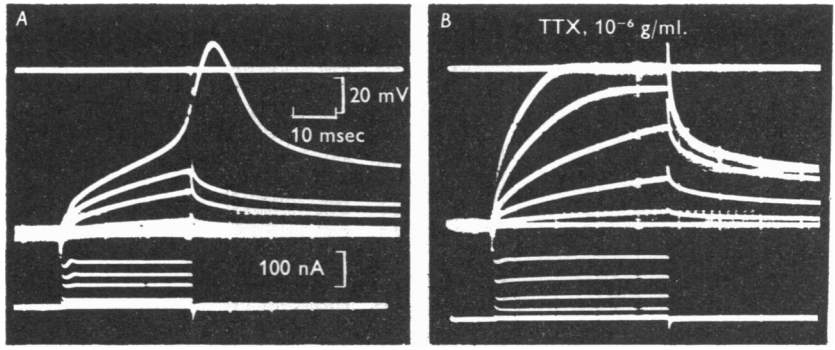


Text-fig. 7. Inactivation of action potential due to membrane depolarization. Slow fibre in a pyriformis 21 days after crushing the sciatic. The resting potential was -69 mV, and the membrane potential was driven to the values indicated. After the records in *D*, the fibre was hyperpolarized and similar action potentials could again be elicited.

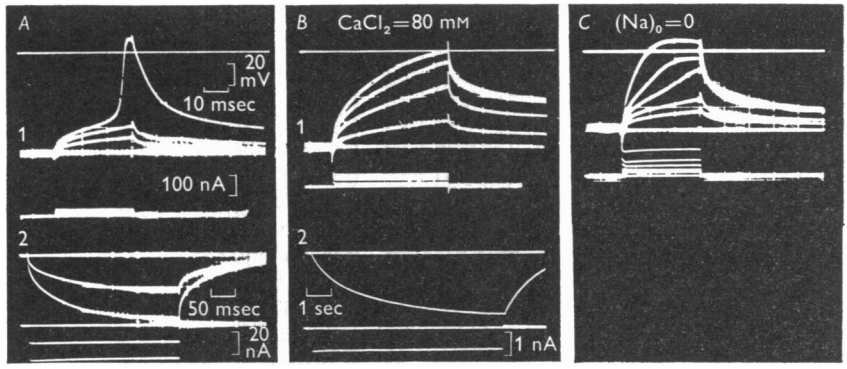
whereas in barnacle muscle fibres the action potential depends on an increased permeability to Ca ions (Hagiwara & Nakajima, 1966) and a regenerative Ca-response is also found in squid and frog nerve terminals (Katz & Miledi, 1969; Miledi, 1971). It was, therefore, of interest to determine the ionic dependence of the action potential system which has been induced in slow muscle fibres.

One way of approaching this question is by using tetrodotoxin, which is known to abolish selectively sodium action potentials in various tissues (see review by Kao, 1966). It was found that action potentials in fast and

slow muscle fibres were abolished when tetrodotoxin (10^{-6} g/ml.) was applied (Text-fig. 8). Further evidence that action potentials in slow muscle fibres are Na-dependent is given in Text-fig. 9 which shows that potentials are also abolished by removing the external Na and replacing it either by Tris or Ca ions. The effects of tetrodotoxin, and of replacing Na ions were similar in slow fibres from denervated muscles to those undergoing re-innervation.



Text-fig. 8. Abolition of the slow fibre action potential by tetrodotoxin (TTX, 10^{-6} g/ml.) *A*: before toxin (E_{RP}: -57 mV; E_m: -90 mV). *B*: approximately 10 min after applying TTX. Pyriformis muscle 13 days after crushing the sciatic.



Text-fig. 9. Na dependence of the action potential in a slow muscle fibre. Pyriformis muscle 13 days after crushing the sciatic. *A*; in normal Ringer. *B*: lack of action potential in isotonic Ca. *C*: lack of action potential after replacement of Na by Tris. E_{RP}: -57 mV in *A*; -85 mV in *B* and -55 mV in *C*. E_m: -92 mV in *A*; -86 mV in *B* and -89 mV in *C*.

DISCUSSION

The central interest of this work lies in the finding that slow muscle fibres acquire the ability to generate action potentials, when they are denervated or when re-innervated by fast axons. There is conclusive

evidence that these fibres are indeed 'slow fibres' because they were characterized not only by their electrical, but also by their ultrastructural features which remained essentially unaltered after the operative procedures.

Action potentials in slow muscle fibres resembled those found in fast fibres, and arose evidently from an increase in Na permeability. Thus, the operative procedure induces in the membrane of slow muscle fibres the appearance of the Na 'channels' which are required for the production of action potentials. It seems that action potentials in slow fibres have smaller amplitudes and slower rates of rise and fall than action potentials in fast fibres. This might indicate that the density of Na sites is still rather less than in fast fibres.

The present study should be compared with the study of slow fibre contractures described elsewhere (Elul *et al.* 1970). Both studies indicate that fast nerve fibres induce changes in slow muscle fibres, namely, loss of maintained contracture and appearance of action potentials. However, one important difference is that while slow fibres retain their maintained contracture response after denervation, denervation alone is sufficient to induce the action potential mechanism.

During re-innervation, slow muscle fibres are innervated first, presumably, by fast axons. During that period the muscle fibres would retain, or perhaps even develop further, their acquired ability to generate action potentials, whilst the maintained contracture response is lost. After long-term re-innervation, slow muscle fibres revert to their normal state: i.e. the fibres lose the action potential mechanism and regain the maintained contracture response, presumably because they have become re-innervated by small axons which then resume the control of the contracture response. A similar restoration of normal function, when cross-innervated fish muscle is re-innervated by its original nerve, has been recently reported by Marotte & Mark (1970).

The development of an action potential mechanism in slow muscle fibres may appear as a dramatic, but not entirely unexpected change in the muscle membrane. It is already known that the molecular arrangements which convey to the muscle membrane its acetylcholine sensitivity (the so-called acetylcholine receptors) are under neural control (Miledi, 1960, 1962; Thesleff, 1960). We know also that acetylcholine receptors are distributed differently in fast and slow twitch fibres of the rat, and that the distribution of receptors in the fibres is determined by the type of innervation they receive (Miledi & Zelena, 1966; Miledi, Stefani & Zelena, 1968).

In analogy with the studies of acetylcholine receptors, it was previously suggested that the control of contracture response in rat and frog slow fibres may depend on a neural influence which regulates calcium-receptors

in the muscle (Miledi & Stefani, 1969; Elul *et al.* 1970). It seems reasonable now to propose that sodium 'receptors' in the slow fibre membrane are also under control of a neural influence. Further evidence that the action potential may be altered by neural influences is provided by Redfern, Lund & Thesleff (1970) who found that the action potential mechanism of rat muscle fibres becomes resistant to tetrodotoxin after denervation.

As a working hypothesis one could assume that the ability to produce an action potential depends on the presence of a macromolecule – say a protein – in the membrane. During embryonic differentiation all muscle fibres may acquire that molecule, and with it the capacity to generate action potentials. With further development, slow muscle fibres would lose the action potential mechanism as they become innervated by the small axons. These axons may suppress the action potential mechanism either because they repress the production of the required molecules, or because they induce the formation of a substance which blocks the action potential mechanism.

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EXPLANATION OF PLATES

PLATE 1

Electron-microscopic structure of an isolated slow muscle fibre capable of generating action potentials. Same fibre as in Text-fig. 2, isolated after the experiment. Glutaraldehyde → OsO_4 fixation. PTA stained. Calibration mark in all Plates = 1 μm.

PLATE 2

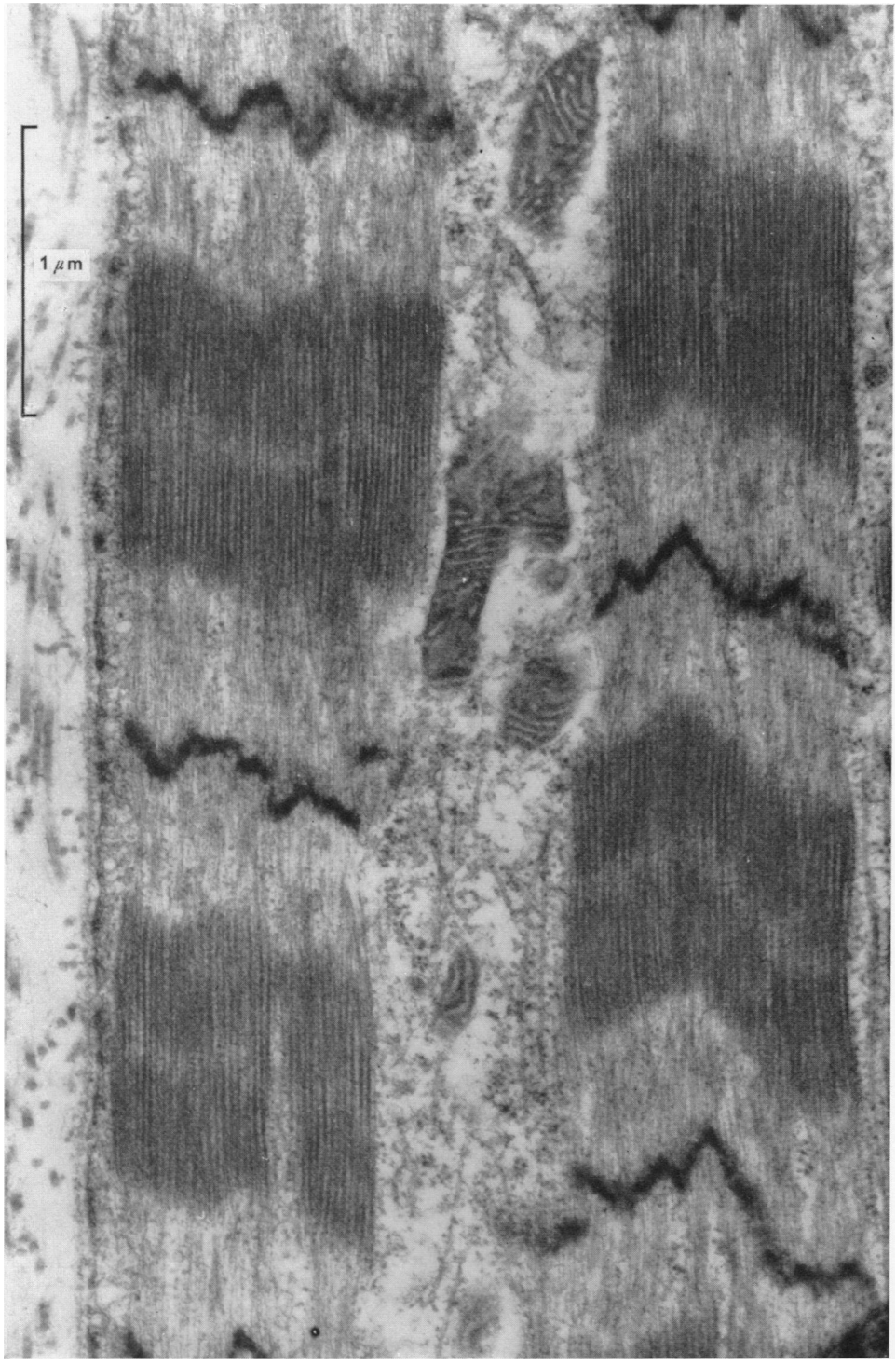
Structure of an isolated slow muscle fibre capable of generating action potentials. From an iliofibularis muscle 32 days after crushing the sciatic. Neuromuscular transmission had not yet been restored. Note the absence of a well defined M-line in the A bands (A).

PLATE 3

Endoplasmic reticulum in a slow fibre capable of generating action potentials. Same fibre as in Pl. 2. Note the fine transverse tubules and filaments (arrows) in the region of the Z-line.

PLATE 4

Another aspect of the same slow fibre as in Pls. 2 and 3.



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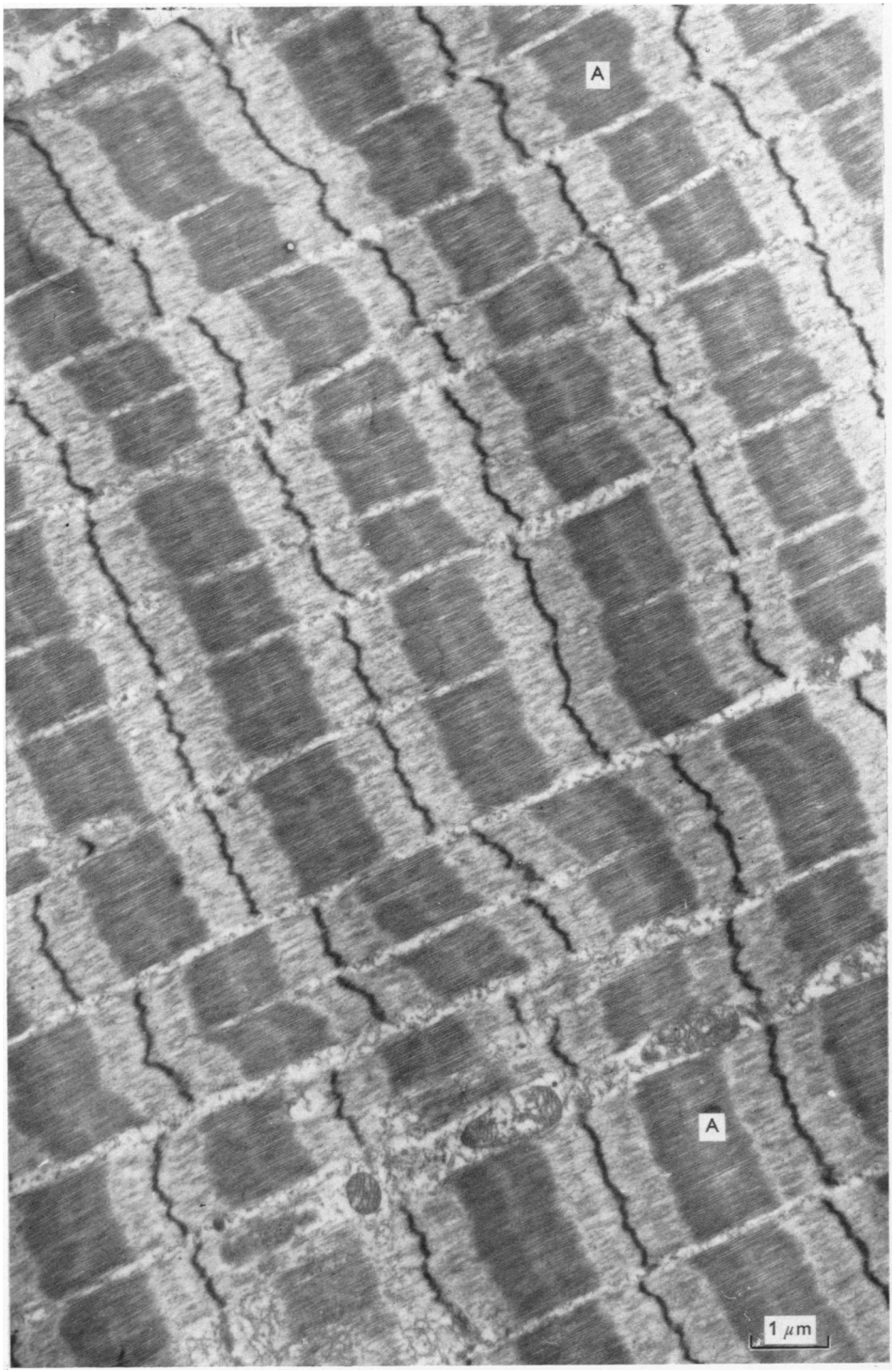

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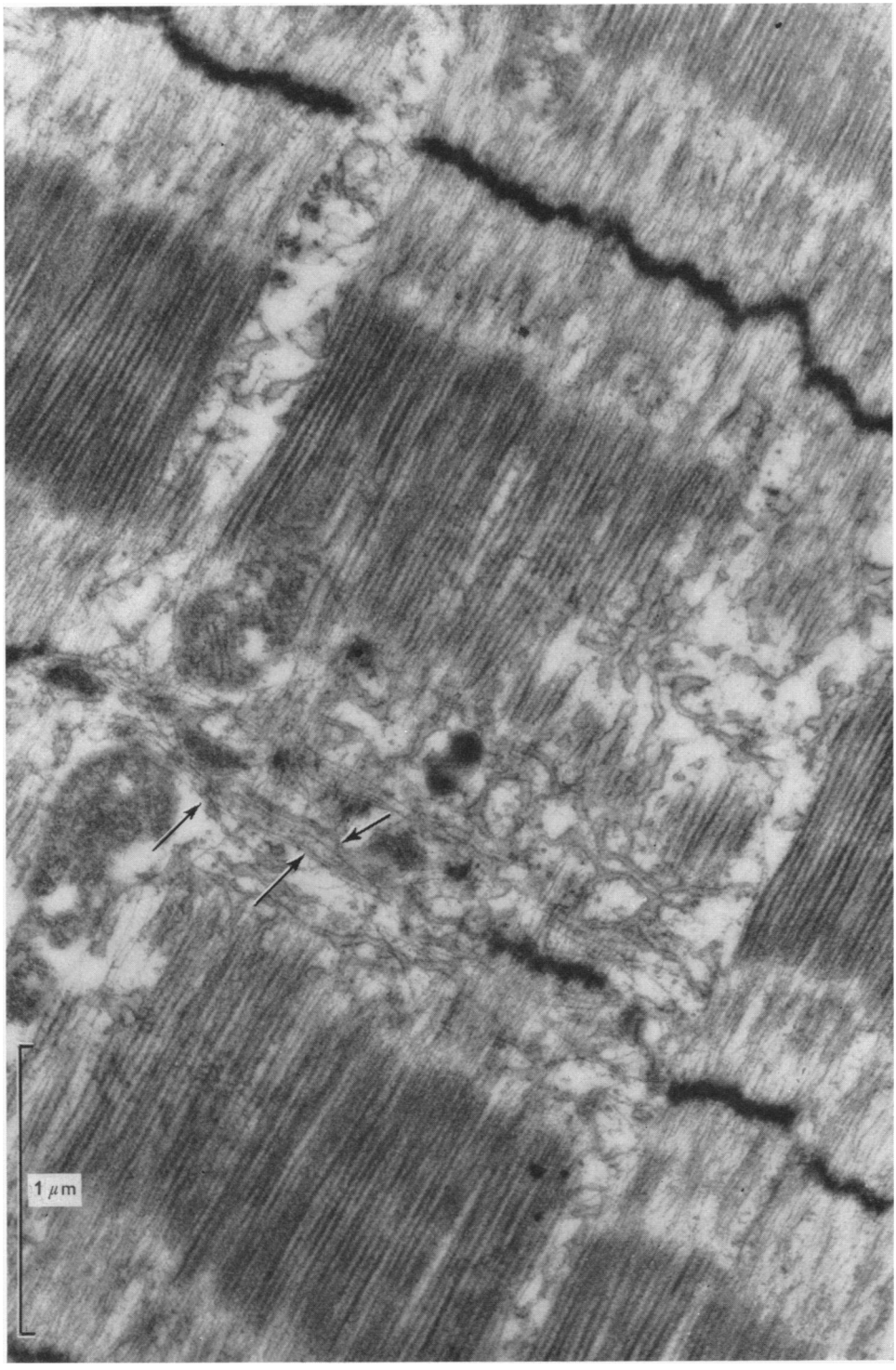

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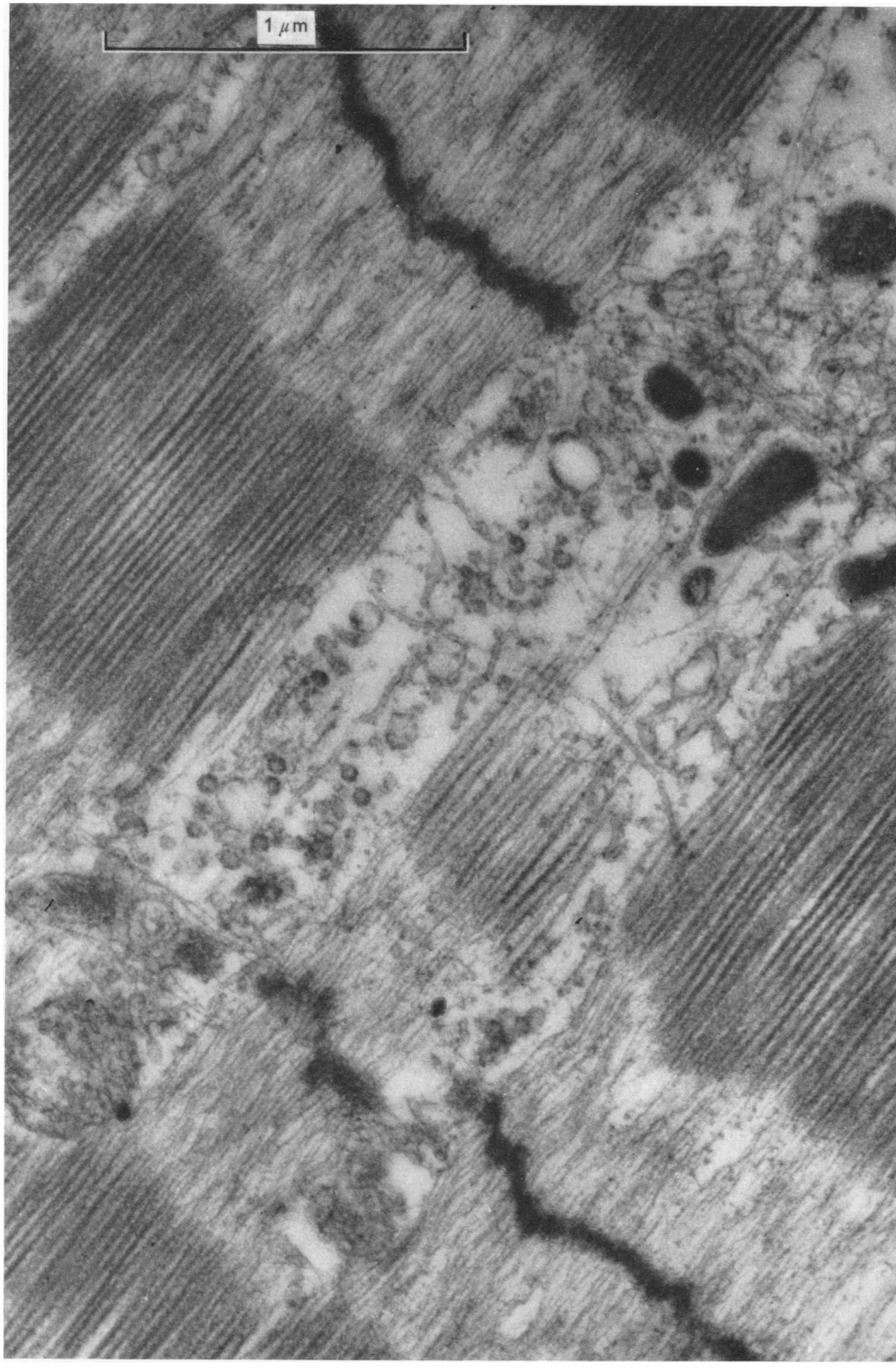

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